DEVELOPMENT OF PLGA NANOPARTICLE INTRANASAL SPRAY OF ESCITALOPRAM FOR THE TREATMENT OF DEPRESSION

SYNOPSIS OF THE THESIS

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DECLARATION

I hereby declare that the dissertation synopsis entitled "Development of PLGA nanoparticles intranasal spray of *Escitalopram* for the treatment of depression" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associateship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

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1.0 INTRODUCTION

1.1 Depression:

Depression is a debilitating mental disorder, characterized by low mood, hopelessness, sadness, low self-esteem, disturbed sleep and often with suicidal thoughts.(1) Depression is the leading cause of disability worldwide and affects more than 350 million people globally.(2) According to WHO, Nowadays, depression is estimated to be the serious disease .(3, 4) Depression is resulting into a form of illness and causing disability in the entire world.(5) Pathological causes of depression include a chemical imbalance in the brain, energy metabolic decline and alteration in body hormones.(6) Depression is not alone responsible for affecting one's life in a destructive way but it also occur in comorbidity with other mental problems such as anxiety disorders and show associated symptoms.(7) Approximately one-third of depressed patients are diagnosed with major depressive disorder (MDD).(8) There are several possible causes of depression. They can range from biological to circumstantial.

1.1.1 Cause of Depression: The primary cause of depression is the disturbances in the monoaminergic i.e. norepinephrine, serotonin, dopamine transmission in brain due to complex interaction of several social, psychological and biological factors.(9) Some other causes are:

- **Brain chemistry.** There may be a chemical imbalance in parts of the brain that manage mood, thoughts, sleep, appetite, and behavior in people who have depression.
- **Family history.** You're at a higher risk for developing depression if you have a family history of depression or another mood disorder.
- **Early childhood trauma.** Some events affect the way your body reacts to fear and stressful situations.
- **Substance use.** A history of substance or alcohol misuse can affect your risk.
- **Pain.** People who feel emotional or chronic physical pain for long periods of time are significantly more likely trusted Source to develop depression.(10)

1.1.2 Symptoms:

Although depression may occur only once during your life, people typically have multiple episodes. During these episodes, symptoms occur most of the day, nearly every day and may include:

- Feelings of sadness, tearfulness, emptiness or hopelessness
- Angry outbursts, irritability or frustration, even over small matters
- Loss of interest or pleasure in most or all normal activities, such as sex, hobbies or sports
- Sleep disturbances, including insomnia or sleeping too much
- Tiredness and lack of energy, so even small tasks take extra effort
- Reduced appetite and weight loss or increased cravings for food and weight gain
- Slowed thinking, speaking or body movements
- Feelings of worthlessness or guilt, fixating on past failures or self-blame
- In younger children, symptoms of depression may include sadness, irritability, clinginess, worry, aches and pains, refusing to go to school, or being underweight.(11)

1.1.3 Mechanism of depression:

Serotonin is synthesized from tryptophan released by vesicular transport. The serotonin transporter is located at the presynaptic neuron and mediates reuptake of serotonin from the synaptic cleft. The signal to the postsynaptic neuron is mediated by receptors (figure-1).

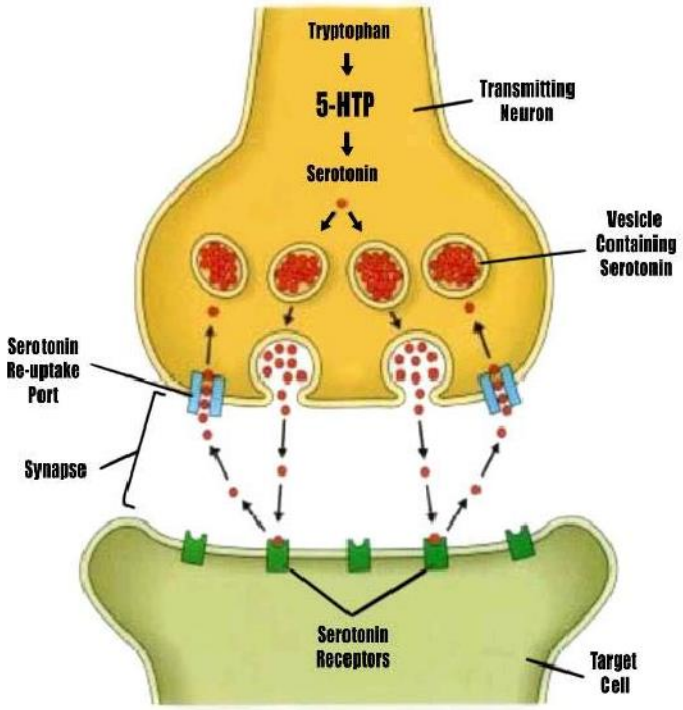


Figure-1: Illustrates the signaling from the presynaptic serotonergic neuron to the postsynaptic neuron mediated by serotonergic neurotransmission.(11)

1.1.4 Treatments of depression:

Several classes of antidepressant therapy that are currently on the market include selective serotonin reuptake inhibitors (SSRI), tricyclic antidepressants, serotonin-norepinephrine reuptake inhibitors, and monoamine oxidase inhibitors.(12) Most of the drugs intended for treatment of depression enhance the availability of monoamines at the synapse by various mechanisms. Nevertheless, the therapeutic efficacy of these antidepressants is dependent on their continuous prolonged presence at the site of action in brain(table no.-1).(13)

Table-1: Approved Antidepressants

Sr. No.	Classifications	Examples
1	Selective serotonin reuptake inhibitors (SSRIs)	Fluoxetine Citalopram Paroxetine Vilazodone
2	Serotonin norepinephrine reuptake inhibitors (SNRIs)	Venlafaxine Duloxetine Desvenlafaxine Levomilnacipran Escitalopram
3	Tricyclic antidepressants (TCAs)	Imipramine Nortriptyline Amitriptyline
4	Monoamine oxidase inhibitors (MAOIs)	Monoamine oxidase inhibitors (MAOIs)
5	Other	Mirtazapine Bupropion

1.2 Nanoparticles:

Recent years have witnessed unprecedented growth of research and applications in the area of nanoscience and nanotechnology. There is increasing optimism that nanotechnology, as applied to medicine, will bring significant advances in the diagnosis and treatment of disease. Anticipated applications in medicine include drug delivery, both in vitro and in vivo diagnostics, nutraceuticals

and production of improved biocompatible materials.(14) Specifically, polymeric nanoparticles (NPs) had become a great subject of research due to their potential as drug carrier, allowing a sustained release of a wide variety of drugs.(15) Additionally, polymeric NPs play a key role in brain drug delivery since they offer several favorable properties. Such NPs are able to incorporate hydrophilic and lipophilic drugs, which then can be delivered by numerous administration routes. Due to their small size, NPs can penetrate the smallest areas of the organism compared with the traditional release systems.(16) Nanoparticles are spherical or nonspherical colloidal structures having particle size of 100–1000 nm. They can be prepared using natural or synthetic polymers. The polymers can be biodegradable or nonbiodegradable.(17)

The primary goals for research of nano-bio-technologies in drug delivery include:

- More specific drug targeting and delivery,
- Reduction in toxicity while maintaining therapeutic effects,
- Greater safety and biocompatibility, and
- Faster development of new safe medicines.(18)

Some of the types of chemical structures and possibilities for the preparation of nanoscale materials used as pharmaceutical carrier system.

Table: 2 Overview of nanoparticles and their application

Particle class	**Materials**	**Application**
materials or Natural derivatives	Chitosan, Dextrane, Gelatine, Alginates, Liposomes, Starch	Drug/Gene delivery
Dendrimers	Branched polymers	Drug delivery
Fullerenes	Carbon based carriers	Photodynamics
Polymer carriers	Polylactic acid, Poly(cyano)acrylates, Polyethyleinemine, Block copolymers, Polycaprolactone	Drug delivery Drug/gene delivery

Various	Silica-nanoparticles	Gene delivery

The shape of NPs (sphere, cube, rod, plate) plays, also, a fundamental role in internalization and drug release processes. Different studies show that spherical particles are good candidates for drug delivery because they can be internalized with high likelihood and faster than rod-shaped NPs(19). The physicochemical features of NPs, such as size, shape, surface charge, surface properties, and conjugation with specific biological molecules, can all be used to improve the delivery of drug to the target tissue through two different ways: passive and active targeting.(20)

1.3 PLGA Nanoparticle:

Poly(D,L-lactic-co-glycolic acid) (PLGA) is one of the most commonly used biodegradable polymer which is usually synthesized by the ring-open co-polymerization of lactide and glycolide.(21) Synthetic polymers and natural macromolecules have been extensively researched as colloidal materials for nanoparticle production designed for drug delivery. Syn- thetic polymers have the advantage of high purity and reproducibility over natural polymers. Among the synthetic polymers, the polyester family (i.e., poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), poly (glycolic acid) (PGA)) are of interest in the biomedical area because of their biocompatibility and biodegradability properties. In particular, poly(lactide-co-glycolide) (PLGA) has been FDA approved for human therapy.(22) Due to its good biocompatibility, excellent safety profile and tunable rate of biodegradation *in vivo*, PLGA has been approved as effective carriers for drug delivery.(22, 23) The size and size distribution of the PLGA nanoparticles among other physical characteristics, are affected by the technique used for the nanoparticle production and the pertinent synthesis parameters, i.e., PLGA molecular mass, the addition of active components, surfactants and other additives.(24-28) Drug release from PLGA particles is achieved through a combination of diffusion and erosion both at the surface and in the bulk, which can be tailored by the physicochemical attributes of PLGA polymers.(29)

1.4 Nasal Drug Delivery Route:

The nasal route has gained attention as it is a direct non-invasive way to transport drugs to the brain which cannot be transferred via the oral route. To date, olfactory and trigeminal nerves have been shown to be safe and effective pathways to deliver therapeutic agents to brain (Figure-2). The olfactory pathway is composed of the olfactory epithelium, lamina propria, and olfactory

bulb. Three types of cells, neuronal cells, progenitor cells, and supporting cells belong to the olfactory epithelium and are connected by tight junctions. An information pathway to the brain is built by neuronal cells which start from the olfactory bulb in the CNS to the olfactory epithelium in the nasal cavity.(30)

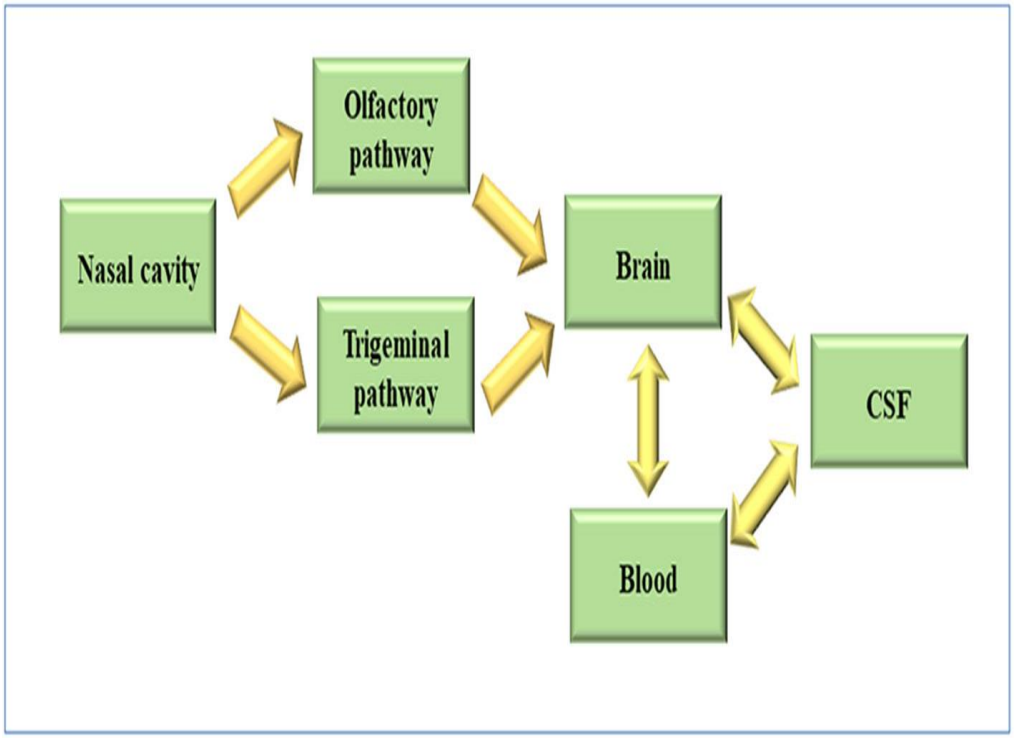


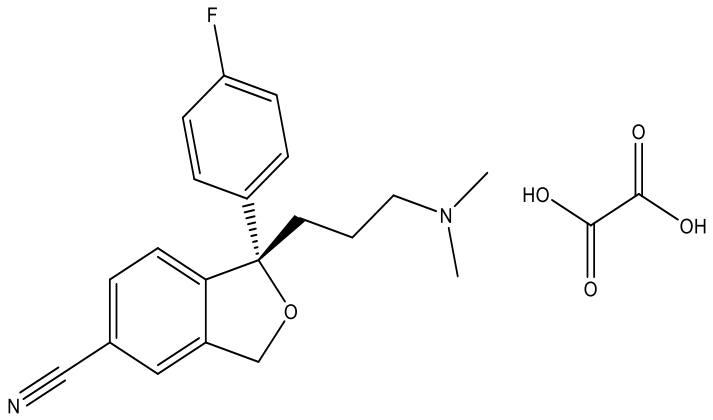
Figure-2: Pathway for brain targeting after intranasal administration.

1.4.1 Advantages of nasal drug delivery system:

- Absorption of drug is rapid via highly vascularised mucosa.
- Availability of large nasal mucosal surface area for dose absorption.
- Onset of action is rapid.
- Non invasive and easy for administration.
- Bypass the BBB.
- Degradation of drug observed in GIT is avoided.
- Hepatic first pass metabolism is absent.
- Nasal bioavailability of small drug molecules is good.
- Improved bioavailability.

- Side effects are reduced due to low dose.(31-33)

1.5 Drug profile:



(Escitalopram)

- Physical form: White to off-whitwe crystalline powder
- Its molecular formula is C20H21FN2O ·
- C2H2O4 with a molecular mass of 414.40.
- Meltng point: 147-155C
- LogP value: 3.76
- Escitalopram is the pharmacologically active S-(+)-enantiomer of racemic citalopram. Escitalopram potentiates serotonergic activity in the central nervous system by selectively binding to human SERT to inhibit 5-HT reuptake.(34)

2.0 REVIEW OF LITERATURE:

Hasler G.et al., 2010; studied the current neurobiological theories with the most valid empirical foundation and the highest clinical relevance are reviewed with respect to their strengths and weaknesses. The selected theories are based on studies investigating psychosocial stress and stress hormones, neurotransmitters such as serotonin, norepinephrine, dopamine, glutamate and gamma-aminobutyric acid (GABA), neurocircuitry, neurotrophic factors, and circadian rhythms. Because all theories of depression apply to only some types of depressed patients but not others, and because depressive pathophysiology may vary considerably across the course of illness, the current extant knowledge argues against a unified hypothesis of depression. As a consequence, antidepressant treatments, including psychological and biological approaches, should be tailored for individual patients and disease states. Individual depression hypotheses based on neurobiological knowledge are discussed in term.

Duncan R. et al., 2003; prepared the nanoparticles interface of polymer chemistry and the biomedical sciences has given rise to the first nano-sized (5-100 nm) polymer-based pharmaceuticals, the 'polymer therapeutics'. Polymer therapeutics include rationally designed macromolecular drugs, polymer-drug and polymer-protein conjugates, polymeric micelles containing covalently bound drug, and polyplexes for DNA delivery. The successful clinical application of polymer-protein conjugates, and promising clinical results arising from trials with polymer-anticancer-drug conjugates, bode well for the future design and development of the ever more sophisticated bio-nanotechnologies that are needed to realize the full potential of the post-genomic age.

Bari NK et al., 2015; prepared, characterization and in vivo evaluation of thiolated chitosan nanoparticles (TCS-NPs) of buspirone hydrochloride (BUH) for brain delivery through intranasal route. TCS NPs were prepared by ionic gelation method and characterized for various parameters. The NPs formed were having particle size of 226.7±2.52nm with PDI 0.483±0.031. Drug entrapment efficiency (EE) and loading capacity (LC) were found to be 81.13±2.8 and 49.67±5.5%. The cumulative percentage drug permeation through nasal mucosa was 76.21%. Bioadhesion study carried out on porcine mucin and showed a bioadhesion efficiency of 90.218±0.134%. Nose-to-brain delivery of placebo NPs was investigated by confocal laser scanning microscopy (CLSM) technique using rhodamine-123 as a marker. The brain concentration achieved after intranasal administration of TCS-NPs was 797.46±35.76ng/ml with

tmax 120min which was significantly higher than achieved after intravenous administration on BUH solution 384.15±13.42ng/ml and tmax of 120min and intranasal administration of BUH solution 417.77±19.24ng/ml and tmax 60min.

Kashi TSJ et al., 2012; The nanoparticles prepared in this study were spherical, with an average particle size of 85-424 nm. The entrapment efficiency of the nanoparticles prepared using different methods was as follows: solid/oil/water ion pairing (29.9%) > oil/oil (5.5%) > water/oil/water (4.7%) > modified oil/water (4.1%) > nano precipitation (0.8%). Addition of dextran sulfate as an ion pairing agent, acting as an ionic spacer between PEGylated PLGA and minocycline, decreased the water solubility of minocycline, hence increasing the drug entrapment efficiency. Entrapment efficiency was also increased when low molecular weight PLGA and high molecular weight dextran sulfate was used. Drug release studies performed in phosphate buffer at pH 7.4 indicated slow release of minocycline from 3 days to several weeks. On antibacterial analysis, the minimum inhibitory concentration and minimum bactericidal concentration of nanoparticles was at least two times lower than that of the free drug.

Danhier F et al., 2012; Poly(lactic-co-glycolic acid) (PLGA) is one of the most successfully developed biodegradable polymers. Among the different polymers developed to formulate polymeric nanoparticles, PLGA has attracted considerable attention due to its attractive properties: (i) biodegradability and biocompatibility, (ii) FDA and European Medicine Agency approval in drug delivery systems for parenteral administration, (iii) well described formulations and methods of production adapted to various types of drugs e.g. hydrophilic or hydrophobic small molecules or macromolecules, (iv) protection of drug from degradation, (v) possibility of sustained release, (vi) possibility to modify surface properties to provide stealthness and/or better interaction with biological materials and (vii) possibility to target nanoparticles to specific organs or cells. This review presents why PLGA has been chosen to design nanoparticles as drug delivery systems in various biomedical applications such as vaccination, cancer, inflammation and other diseases. This review focuses on the understanding of specific characteristics exploited by PLGA-based nanoparticles to target a specific organ or tissue or specific cells.

Borm P et al., 2006; describes The use of nanotechnology in medicine and more specifically drug delivery is set to spread rapidly. Currently many substances are under investigation for drug delivery and more specifically for cancer therapy. Interestingly pharmaceutical sciences are using nanoparticles to reduce toxicity and side effects of drugs and up to recently did not realize that

carrier systems themselves may impose risks to the patient. The kind of hazards that are introduced by using nanoparticles for drug delivery are beyond that posed by conventional hazards imposed by chemicals in classical delivery matrices. For nanoparticles the knowledge on particle toxicity as obtained in inhalation toxicity shows the way how to investigate the potential hazards of nanoparticles. The toxicology of particulate matter differs from toxicology of substances as the composing chemical(s) may or may not be soluble in biological matrices, thus influencing greatly the potential exposure of various internal organs. This may vary from a rather high local exposure in the lungs and a low or neglectable exposure for other organ systems after inhalation. However, absorbed species may also influence the potential toxicity of the inhaled particles. For nanoparticles the situation is different as their size opens the potential for crossing the vari- ous biological barriers within the body. From a positive viewpoint, especially the potential to cross the blood brain barrier may open new ways for drug delivery into the brain. In addition, the nanosize also allows for access into the cell and various cellular compartments including the nucleus. A multitude of substances are currently under investigation for the preparation of nanoparticles for drug delivery, varying from biological substances like albumin, gelatine and phospholipids for liposomes, and more substances of a chemical nature like various polymers and solid metal containing nanoparticles.

Chithrani BD et al., 2006; Investigate the intracellular uptake of different sized and shaped colloidal gold nanoparticles. We showed that kinetics and saturation concentrations are highly dependent upon the physical dimensions of the nanoparticles (e.g., uptake half-life of 14, 50, and 74 nm nanoparticles is 2.10, 1.90, and 2.24 h, respectively). The findings from this study will have implications in the chemical design of nanostructures for biomedical applications (e.g., tuning intracellular delivery rates and amounts by nanoscale dimensions and engineering complex, multifunctional nanostructures for imaging and therapeutics).

Matsumura Y et al., 1986; Determined the general mechanism of this tumoritropic accumulation of smancs and other proteins, we used radioactive (51Cr-labeled) proteins of various molecular sizes (Mr 12,000 to 160,000) and other properties. In addition, we used dye-complexed serum albumin to visualize the accumulation in tumors of tumor-bearing mice. Many proteins progressively accumulated in the tumor tissues of these mice, and a ratio of the protein concentration in the tumor to that in the blood of 5 was obtained within 19 to 72 h. A large protein like immunoglobulin G required a longer time to reach this value of 5. The protein

concentration ratio in the tumor to that in the blood of neither 1 nor 5 was achieved with neocarzinostatin, a representative of a small protein (Mr 12,000) in all time. We speculate that the tumoritropic accumulation of these proteins resulted because of the hypervasculature, an enhanced permeability to even macromolecules, and little recovery through either blood vessels or lymphatic vessels. This accumulation of macromolecules in the tumor was also found after i.v. injection of an albumin-dye complex (Mr 69,000), as well as after injection into normal and tumor tissues. The complex was retained only by tumor tissue for prolonged periods. There was little lymphatic recovery of macromolecules from tumor tissue. The present finding is of potential value in macromolecular tumor therapeutics and diagnosis.

Qodratnama R et al.,2015; performed A novel delivery system, capable of providing both structural support and controlled release kinetics, has been developed from PLGA microparticles. The inclusion of a hydrophilic PLGA–PEG–PLGA triblock copolymer altered release kinetics such that they were decoupled from polymer degradation. A quasi zero order release profile over four weeks was produced using 10% w/w PLGA–PEG–PLGA with 50:50 PLGA whereas complete and sustained release was achieved over ten days using 30% w/w PLGA–PEG–PLGA with 85:15 PLGA and over four days using 30% w/w PLGA–PEG–PLGA with 50:50 PLGA. These three formulations are promising candidates for delivery of growth factors such as BMP-2, PDGF and VEGF. Release profiles were also modified by mixing microparticles of two different formulations providing another route, not previously reported, for controlling release kinetics. This system provides customisable, localised and controlled delivery with adjustable release profiles, which will improve the efficacy and safety of recombinant growth factor delivery.

Makadia HK et al., 2011; PLGA is biocompatible and biodegradable, exhibits a wide range of erosion times, has tunable mechanical properties and most importantly, is a FDA approved polymer. In particular, PLGA has been extensively studied for the development of devices for controlled delivery of small molecule drugs, proteins and other macromolecules in commercial use and in research. This manuscript describes the various fabrication techniques for these devices and the factors affecting their degradation and drug release.

Leopold DA.et al.,1988; studied the relationship between nasal anatomy and olfactory ability was evaluated by correlating measurements from nasal cavity CT scans with the results of an established clinical measure of olfactory function (Odorant Confusion Matrix, or OCM). The

most appropriate mathematical model of this relationship was developed by analyzing the logarithms of 30 anatomical measures and a logistic transform of the OCM percent correct score. Two nasal cavity regions were found to be the most important factors in accounting for the olfactory test results, with a third region modifying the effects of the first two. The results of this study suggest that nasal anatomy may play a role in controlling the access of an odorant to the olfactory receptor area. The conclusions of this study have implications for nasal surgeons who, when possible, should make attempts to repair or preserve these nasal regions which seem to be so important to olfactory function.

Singh AK et al.,2012; studied the drug delivery through nasal route has attracted the interest of scientific community as it has been potentially explored as an alternative route for the administration of vaccines and biomolecules such as proteins, peptides and non-peptide drugs that are susceptible to enzymatic or acidic degradation and first-pass hepatic metabolism. The nasal mucosa is one of the most permeable and highly vascularized site for drug administration ensuring rapid absorption and onset of therapeutic action. In addition it minimizes the lag time associated with oral drug delivery and offers noninvasiveness, self-medication, patient comfort and patient compliance which are hurdled in intravenous drug therapy. The objective of this review is to provide an anatomical, histological and physiological overview of nose, absorption enhancers, barriers related to nasal drug delivery, physicochemical, biological and formulation related factors affecting nasal drug delivery system and its advantages. It also highlights research approaches on brain targeting through nasal cavity.

Owens MJ,et al.,2001; studied that escitalopram inhibited 5-HTT functions approximately 2 times more potently than citalopram and at least 40 times more potently than R-citalopram. Escitalopram showed insignificant activity at other monoamine transporters and 144 other binding sites. Escitalopram inhibited 5-HT neuronal firing in DRN and potentiated 5-HTP-induced behaviours more potently than citalopram; R-citalopram was inactive. Escitalopram and citalopram, but not R-citalopram, reduced forced-swimming-induced immobility and facilitated exploratory behaviour in the black and white box. Escitalopram and citalopram inhibited USV potently; R-citalopram was several times less potent. Escitalopram, citalopram and R-citalopram inhibited aggressive behaviour weakly. Escitalopram and citalopram had very potent anti-aggressive effects when co-administered with l-5-HTP.

Rawat M,et al.,2009; formulate and effect of external aqueous phase volume was found to be higher on the entrapment efficiency of microspheres (SSY_1 = 1362.63; SSY_2 = 250.13), whereas Tween® 80 produced a significant effect on size of microspheres (SSY_1 = 944.01; SSY_2 = 737.26). Scanning electron microscopy of microspheres demonstrated smooth surface spherical particles. The effect of formulation variables on the integrity of enzyme was confirmed by *in vitro* proteolytic activity. Microspheres having maximum drug encapsulation (81.32 ± 3.97) released 4–5% enzyme at pH 1.2 in 2 h. The release of enzyme from microspheres followed Higuchi kinetics (R^2 = 0.987). In phosphate buffer, microspheres showed an initial burst release of 25.65 ± 2.35% in 1 h with an additional 62.96 ± 4.09% release in the next 5 h. Thus, formulation optimization represents an economical approach for successful preparation of Eudragit S100 microspheres involving fewest numbers of experiments.

Ujwala K.,2020; developed a novel technique for selecting hydrotropic agents to enhance the solubility of low soluble drug, paroxetine hydrochloride. Paroxetine hydrochloride was estimated using UV spectrophotometry at 293 nm and hydrotropic agents did not interfere with drug estimation. In order to elucidate the interaction, a UV spectrophotometric method was developed. As the concentration of the hydrotropic agent increased, the solubility of paroxetine hydrochloride increased linearly for nicotinamide, citric acid and tartaric acid at 25° and 37°. Neutralization by pH adjustment technique was employed with hydrotropic agents to prepare hydrotropes. Each hydrotrope was characterized by melting point, Fourier transform infrared spectroscopy, solubility and dissolution. The *in vitro* dissolution studies showed that paroxetine hydrochloride release was enhanced from the prepared hydrotropes (>80%) in comparison to the pure drug (37%) in 40 min. *In vitro* dissolution studies of tablets prepared using hydrotropes demonstrated two times increase in drug release (>88%) in comparison to the marketed tablets (56%) in 40 min.

Sharma S., 2020; performed the paroxetine (Par) is an antidepressant (selective serotonin reuptake inhibitor) used for the treatment of depression and anxiety related problems, but due to its degradation in first pass metabolism it suffers from the problem of poor oral bioavailability (less than 50%). The present study focuses on developing a paroxetine loaded PLGA (Poly (lactic-co-glycolic acid) nanoparticles (NPs) for effective delivery. The nanoparticles were developed by nanoprecipitation method and the mean particle size of the Par-PLGA-NPs was found to be 184.20 ± 1.1 nm with polydispersity index of 0.187 and zeta potential was observed

to be in the range of -25.6 ± 0.43 mV. Entrapment efficiency (EE %) of formulated Par-PLGA-NPs was 86.03% with drug loading of 14.06%. In vitro, cytotoxicity was evaluated using MTT assay and result indicated that cells were more viable i.e. 93.30% when treated with Par-PLGA-NPs as compared to cells treated with Par aqueous solution. Thus, it can be concluded that the developed Par-PLGA-NPs provide a possible substitute for existing drug formulation of paroxetine.

Nigam K.,2019; performed nose-to-brain delivery of drugs and faster onset of action have made intra-nasal route a much sought-after alternative to conventional routes of drug delivery to the brain. Lamotrigine is used for the treatment and management of neuropathic pain, and in the present work, lamotrigine (LTG)-PLGA nanoparticles were developed for intra-nasal delivery. The LTG-PLGA nanoparticles were prepared using modified nanoprecipitation method via high-speed homogenization and ultra-sonication techniques. Entrapment efficiency (EE%) of developed LTG-PLGA-NPs was found to be $84.87 \pm 1.2\%$ with drug loading of $10.21 \pm 0.89\%$. The particle size of developed nanoparticles was found to be 184.6 nm with PDI value of 0.082 and zeta potential of -18.8 mV. Dissolution profiles were studied in PBS (pH 7.4), simulated nasal fluid, and simulated cerebrospinal fluid where almost complete release was observed within 5 h in CSF. In vitro, cytotoxicity was analyzed using MTT assay where dose-dependent cytotoxicity was observed for developed LTG-PLGA-NPs. In vitro cytokine analysis showed positive effects of LTG-PLGA-NPs as pro-inflammatory cytokine suppressors. Further, in vivo studies were performed for radiolabeled formulation and drug (^{99m}Tc-LTG-PLGA-NPs and ^{99m}Tc-LTG-aqueous) using Sprague Dawley rats where with the help of gamma scintigraphy studies, various routes of administration viz. oral, intra-nasal, and intra-venous were compared. Various pharmacokinetic parameters were evaluated using biodistribution studies to estimate the drug levels in blood and brain. For ^{99m}Tc-LTG-PLGA-NPs via intra-nasal route, drug targeting efficiency (DTE%) was found to be 129.81% and drug target organ transport (DTP%) to be 22.81% in brain with C_{max} of 3.82%/g within T_{max} 1.5 h. Thus, the developed PLGA nanoparticles for intra-nasal delivery provide a possible alternative for existing available drug formulation for neuropathic pain management.

Lungare S., 2016; studdied that to develop and characterize an intranasal delivery system for amantadine hydrochloride (AMT). Optimal formulations consisted of a thermosensitive polymer Pluronic® 127 and either carboxymethyl cellulose or chitosan which demonstrated gel transition

at nasal cavity temperatures (34 ± 1°C). Rheologically, the loss tangent (Tan δ) confirmed a 3-stage gelation phenomena at 34 ± 1°C and non-Newtonian behavior. Storage of optimized formulation carboxymethyl cellulose and optimal formulation chitosan at 4°C for 8 weeks resulted in repeatable release profiles at 34°C when sampled, with a Fickian mechanism earlier on but moving toward anomalous transport by week 8. Polymers (Pluronic® 127, carboxymethyl cellulose, and chitosan) demonstrated no significant cellular toxicity to human nasal epithelial cells up to 4 mg/mL and up to 1 mM for AMT (IC_{50}: 4.5 ± 0.05 mM). Optimized formulation carboxymethyl cellulose and optimal formulation chitosan demonstrated slower release across an *in vitro* human nasal airway model (43%-44% vs 79 ± 4.58% for AMT). Using a human nasal cast model, deposition into the olfactory regions (potential nose-to-brain) was demonstrated on nozzle insertion (5 mm), whereas tilting of the head forward (15°) resulted in greater deposition in the bulk of the nasal cavity.

3.0 AIM

Depression is the leading cause of disability worldwide and affects more than 350 million people globally. It is categorized by many symptoms like feeling hopelessness or inappropriate guilt, low energy, change in sleep, change in appetite, and recurrent thoughts of suicide. Depression can affect the patient with their whole society . Depression mainly occurred due to the deficiency of both serotonin and norepinephrine neurotransmitters in the brain. Selective serotonin reuptake inhibitors (SSRIs) are those which preferably inhibit 5-HT uptake and which at same time have no or only slight effects on other uptake mechanisms.There are five SSRIs are marketed i.e citalopram, fluoxetine, fluoxemine, paroxetine and sertraline. In these all five drugs citalopram is most selective 5HT uptake inhibitor Escitalopram is the pure S-enantiomer of racemic citalopram that more potently inhibits the serotonin transporter protein (SERT) in comparison to citalopram. Escitalopram has low side effects than other classes of antidepressants. The development of nanoparticles for escitalopram might be useful for increased therapeutic efficacy. The aim of this study was to formulate and evaluate the escitalopram-loaded Poly (D,L-Lactide-co-glycolide) (PLGA). Escitalopram loaded PLGA nanoparticles is prepare by emulsion solvent evaporation technique and optimized for various physiochemical characteristics. Among the different polymers developed to formulate polymeric nanoparticles, PLGA has attracted considerable attention due to its attractive properties i.e biodegradability and biocompatibility. Further, the levels of escitalopram in brain and blood plasma is determine at various time intervals for calculation of different pharmacokinetic parameters. Escitalopram loaded PLGA nanoparticles on intranasal administration significantly reduced the symptoms of depression. Thus, intranasal mucoadhesive escitaloprm PLGA nanoparticles could be potentially used for the treatment of depression.

OBJECTIVE

- Development of PLGA –loaded nanoparticles of Escitolapram.
- Characterization of PLGA –loaded nanoparticles.
- Determination of encapsulation efficiency of Escitalopram PLGA-loaded nanoparticle.
- Formulation of intranasal spray.
- Evaluation of antidepressant potential of Estilopram loaded PLGA nanoaprticles.

4.0 PLAN OF WORK

Literature survey

Procurement of drug and chemicals

Pre-formulation Study

Preparation of PLGA-loaded nanoparticles

Characterization of PLGA-loaded nanoparticles

Formulation of nasal spray

Evaluation of nasal spray

Biological and pharmacokinetic(specific inhalation mask) activity

5.0 METHODOLOGY

5.1 Preparation of PLGA-loaded nanoparticles:

5.1.1 Emulsion solvent evaporation method:

PLGA nanoparticles will be prepare by emulsion solvent evaporation method.(35) Briefly 100 mg of PLGA will dissolve in 2.5 mL of chloroform with escitalopram (30% w/w) and added to 10 ml aqueous phase (0.5% acetic acid solution with pH range of 4.6-4.8) containing 0.5% PVA. The primary emulsion will be vortex for 90 sec and then sonicate by a probe sonicator at 50W for 60 sec on ice. The chloroform will remove by rotary evaporator under partial vacuum. The nanoparticles will be obtaine by ultracentrifugation (23000 x g, 18 min at 4^{o}C. The concentrated nanoparticles will be freeze dried using mannitol as cryoprotectant (2.5% w/w).

5.2 UV-spectrophotometric analysis of Escitalopram:

For the detection of escitalopram double beam spectrophotometer is use with UV-Probe software. To obtain the standard plot for escitalopram, firstly.(36)

5.3 Characterization of PLGA-loaded nanoparticles

5.3.1 Particle size and PDI:

The prepared escitalopram nanoparticles size and polydispersity index is obtain using a photon correlation spectrometer.(37)

5.3.2 Zeta potential:

The surface charge of the nanoparticles will be study to determine the surface charge of nanoparticles and is perform by a zeta potential measuring instrument.(38)

5.3.3 Encapsulation efficiency & drug loading capacity percentage:

The prepared escitalopram PLGA-NPs is centrifuge at 15,000 rpm. The obtained supernatant will taken in separate tube, and absorbance of the suspension is measure using UV-spectrophotometer and the amount of unentrapped drug is measure by determining the amount of escitalopram drug. Drug loading is calculate by weighing the pellet after washing .(39)

5.4 Formulation of nasal spray:

Formulations is initially prepare by a modified 'Cold method'.(40)

5.5 Evaluation of nasal spray

5.5.1 Sol-Gel Transition:

A modified method is used to determine the sol-gel transition temperature (Tsol-gel). A sample of each formulation (1 g) is transfer to a glass vial and heat in a dry block, initiate at 20 °C and increase it by 1 °C after every 5 minutes of equilibration time. The Tsol-gel point is define as the

temperature whereby the upper meniscus of the gel did not move upon tilting the vial by 90 ° and this is use to demark optimal formulations. The optimal subset of formulations is then further characterise.(41)

5.5.2 Rheologic Characterization:

To assess the rheological properties of the formulations, rheological measurements will be determine by AR-G2 Rheometer.

5.5.3 Droplet Size Distribution:

To assess the potential in vivo deposition characteristics of the proposed nasal formulation, a multidose pump spray delivery system will be use to characterize the formulation droplet size distribution using a laser diffraction technique.(42)

5.5 Biological activity:

5.5.1 Stress induced model.

- **Forced swimming test**.(43)

5.6. Drug induced model:

- **Reserpine reversal test (RRT)**.(44)

5.6.3 Biochemical estimation of serotonin, noradrenaline and dopamine.(44)

5.6.4 Blood and brain pharmacokinetics(specific inhalation mask)(45).

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